Using copper more effectively in winemaking


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Winemakers commonly add copper to wines before packaging to remove unpleasant 'reductive' aromas. However, residual copper in wine can have negative consequences including unsightly deposits, increased risk of oxidation and the formation of further reductive compounds. This article reports a pilot study examining the effectiveness of making copper additions during fermentation and using the yeast present to bind up and remove the added copper from the final wine.

AT A GLANCE

- Copper sulfate is commonly used to treat 'reductive' aromas in winemaking
- Additions are often made to finished wine shortly before packaging, which can leave residual copper in packaged wine
- Metal ions in wine can cause oxidation, reduction and haze issues
- Recent experiments investigated the impact of copper additions made at different times on fermentation performance, sulfide concentration and residual copper levels
- Copper additions up to 20mg/L added at the start of fermentation had no impact on fermentation kinetics
- Copper additions made at 0°Brix (towards the end of active ferment) did not affect fermentation performance
- In general, earlier additions resulted in lower residual copper levels
- Additions of 5mg/L at 0°Brix appear to be effective at removing reductive aromas without leaving copper in the final wine
- Further work is needed to focus on the sensory impacts of copper addition levels and timing.

INTRODUCTION

During fermentation volatile sulfur-containing compounds are formed that can have a negative influence on the sensory characters of finished wines (Siebert *et al.* 2010, Spiropoulos *et al.* 2000). Hydrogen sulfide (H_2S), methanethiol (MeSH), ethanethiol (EtSH) and dimethyl sulfide (DMS) are among the most significant of these unpleasant aroma compounds, but many others exist. All of these compounds have low odour detection thresholds, so they have a sensory impact even at very low levels (Siebert *et al.* 2010).

Copper sulfate is commonly added to wine to remove these unpleasant sulfidic wine aromas (often referred to as 'reductive' characters). It has been assumed that the copper ions bind to sulfur-containing compounds to form insoluble copper sulfides (Godden 2000), which are then removed by cold settling or filtration, although some recent work has highlighted the difficulty of this process (Clark *et al.* 2015). Often copper additions

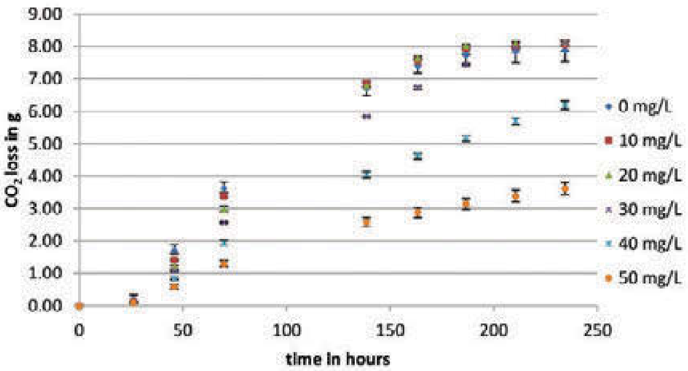

Figure 1. Comparison of fermentation performance for laboratory-scale ferments of Chardonnay juice with different levels of copper (0 to 50mg/L) added at the beginning of fermentation.

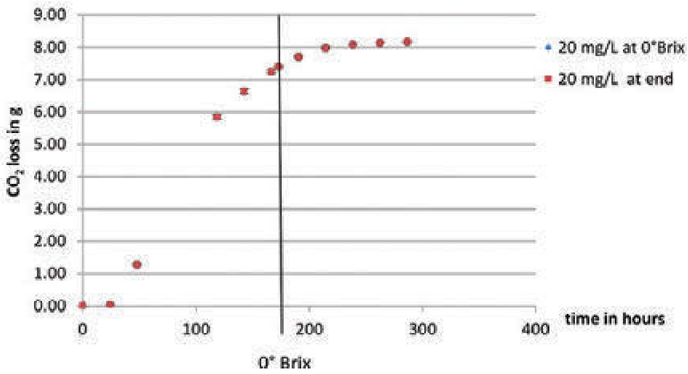

Figure 2. Comparison of fermentation performance for laboratory-scale ferments of Chardonnay juice with 20mg/L copper added at 0°Brix or at the end of fermentation.

are made to finished wines just prior to bottling; however, making additions at this point can result in significant amounts of residual copper in the wine. Also, while copper is very effective at removing H_2S and simple thiols, its ability to remove the more complex sulfur-based reductive compounds that form over time in wine is limited, which means that earlier additions are likely to be more effective.

Fining with $CuSO_4$ is an important tool in the wine industry, but it also has disadvantages. When removing reductive aromas from wine, copper fining can also remove important positive aroma compounds responsible for fruity and tropical characters (Darriet *et al.* 2001). Residual copper also plays a critical role in both oxidative and reductive processes in wine post-packaging. Effects of residual copper can include increased rates of oxidation, loss of beneficial thiols and, perversely, given the reason for its addition, increased formation of H_2S and other negative sulfur-containing compounds (Danilewicz 2007, Viviers *et al.* 2013). To prevent such negative effects it is important to keep residual copper concentrations in packaged wine as low as possible.

USING YEAST TO BIND METALS

One possible way of achieving low residual copper concentrations is to use active yeast cells to bind up copper during fermentation. While the mechanisms for this process (known as biosorption) are not fully understood, some of the possibilities are discussed below.

The flocculation behaviour of some yeast strains may be connected to proteins on the cell surface which might be able to interact with cations such as copper. Lectin is considered to be the protein most responsible for flocculation effects. The best understood mechanism for copper uptake is through its binding via ion exchange with functional groups on the cell walls. It has been shown that amino, carboxyl, phosphate, phosphodiester and hydroxyl groups play an important role in complexation of heavy metal ions (Wang 2006). After longer contact times the copper ions can be taken into the cells and accumulated (Huang *et al.* 1990). Copper can be accumulated by formation of inclusion bodies or by binding to proteins that either mainly contain acid phosphatase or that relate to metal binding proteins. Metallothionein is a cysteine-rich metal-binding protein present in yeast, known for its ability to bind copper (Wang 2006).

This possibility of using yeast's affinity for metals as a way to avoid residual copper in wine after copper fining has been explored in a recent series of experiments at the AWRI. The work aimed to investigate the ability of copper additions made during fermentation to remove H_2S and other sulfides while limiting the levels of residual copper in the finished wines. A series of 200mL laboratory-scale ferments were carried out in which timing and addition rate of copper were varied. Ferments were conducted in Chardonnay and Shiraz juice in triplicate using the AWRI838 yeast strain. Copper addition rates ranged from 5 to 50mg/L depending on the trial. These rates are well above those used by winemakers (usually less than 5mg/L) but were chosen to test the effects at extremes of performance.

DID COPPER ADDITIONS AFFECT FERMENTATION KINETICS?

To assess fermentation performance, the weight loss of each ferment was measured at least once per day and expressed as CO_2 loss per 100g of juice. Copper concentrations up to 40mg/L for Shiraz and up to 20mg/L for Chardonnay added at the beginning of fermentation were found to have little influence on the fermentation kinetics (Figure 1). These concentrations did not appear to be toxic for *S. cerevisiae*. The results are in agreement with those found by other researchers including Azenha *et al.* (2000) and Liang and Zhou (2007). It is important to note, however, that while

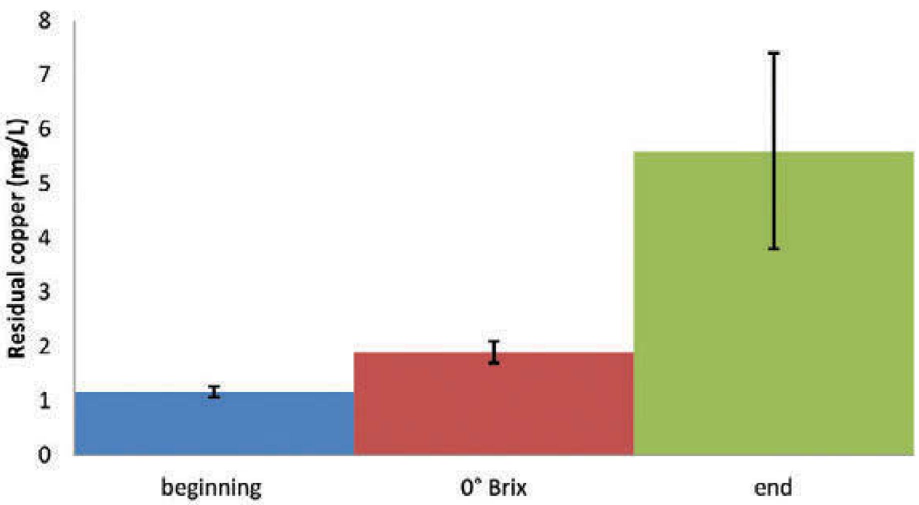

Figure 3. Effect of timing of 10mg/L copper addition on final residual copper concentration.

these additions did not affect fermentation kinetics, the experiment did not investigate if there were any significant taste implications from having such high levels of copper present for the duration of the ferment.

A second experiment investigated the impact of the timing of the copper addition on fermentation kinetics, comparing a 20mg/L addition made at 0°Brix (just before the end of active fermentation) with an addition made after the completion of fermentation. No impact on fermentation performance or the ability of the ferment to finish was caused by the addition at 0°Brix (Figure 2)

DOES TIMING AFFECT RESIDUAL COPPER CONCENTRATION?

A further experiment looked at the influence of addition timing on the final copper concentration in wine by comparing additions of 10mg/L copper to Chardonnay ferments at the beginning of ferment, at 0°Brix and at the end of the fermentation (Figure 3).

Earlier additions of the copper led to lower final copper concentrations. A possible explanation is that the later copper is added, the more likely it will only be absorbed by the cell surface rather than transferred into the interior of the cell. While copper absorbed by the surface of the cell is still removed from solution, it is possible that it may be more available for further reactions if the wine is not promptly removed from lees. The difference between additions at the beginning of ferment and at 0°Brix was much less pronounced than between 0°Brix and the end of ferment.

This may be an important finding as there is still much to be learned about other impacts of copper that could mean additions towards the end of fermentation would be advantageous.

The experiments tended to indicate that yeast was able to remove a consistent amount of copper, independent of the initial copper concentration. This would suggest that to minimise the risk of residual copper in finished wines, it appears prudent to limit copper additions to 5mg/L or less. While not directly tested in this study, this is a target for future work.

IMPACTS ON SULFIDE GENERATION AND SENSORY CHARACTERISTICS

Although copper additions as high as 20mg/L did not affect fermentation performance, lower concentrations were found to be sufficient to remove negative sulfidic aromas without leading to unwanted residual copper concentrations. The amounts of H_2S released during fermentation are not significantly affected by copper additions at 0°Brix as the yeast produce this compound earlier in the fermentation, but it seems that the dissolved H_2S concentration remaining in the wine after fermentation is reduced even by copper additions as low as 5mg/L added at 0°Brix, regardless of the levels produced during fermentation. Interestingly, additions at the beginning of ferment seemed to have a less significant impact on dissolved H_2S in the finished wine (Figure 4). This may be a consequence of the copper being translocated to the interior of the cells

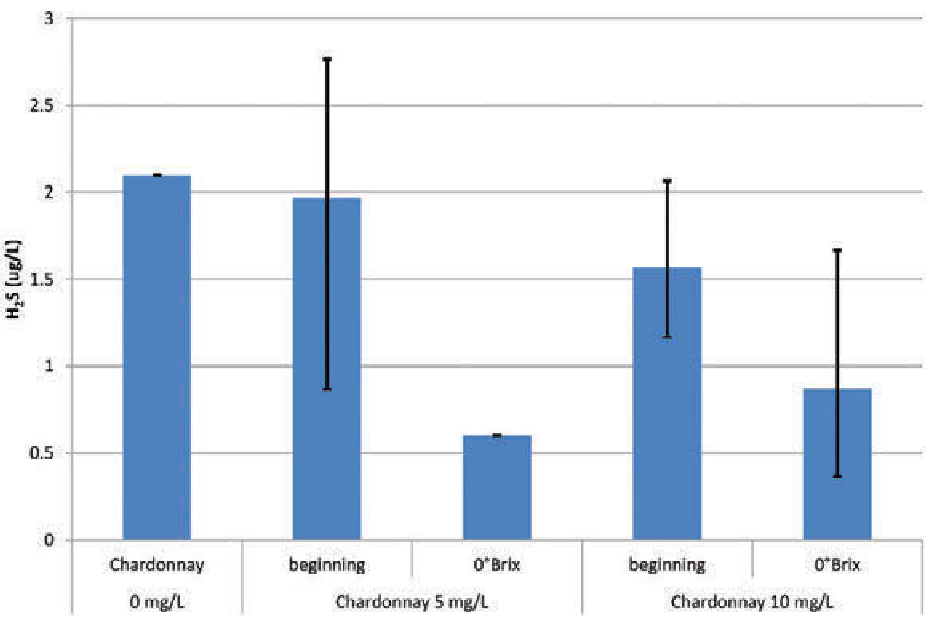

Figure 4. Dissolved H_2S measured post-fermentation in laboratory-scale ferments after experiments comparing copper additions of 5mg/L and 10mg/L at the beginning of ferment and at 0°Brix with an untreated control.

early in ferment, or perhaps because the copper was scavenging sulfides that would normally have been carried away by fermentation gases, leaving less copper available to interact with residual dissolved sulfides.

Limited sensory evaluations of Chardonnay wines from the trial showed that copper-treated wines differed in their aromas from control wines, independent of how much copper was added. It was, however, confirmed that it may be better to make copper additions at 0°Brix rather than at the beginning of fermentation to minimise the impacts on positive sensory characters.

CONCLUSIONS

The current trial suggests that copper additions of 5mg/L or less at 0°Brix are suitable to treat wines that show reductive aromas. Such additions are unlikely to result in increased residual copper in the final wine as long as the wine is removed from gross lees shortly after ferment.

Further work is needed to understand the sensory implications of copper additions of different magnitudes and at different times. It would be interesting to see if copper concentrations below 5mg/L are effective at removing reductive aromas when added at 0°Brix. If so, it may be possible to remove or reduce sulfidic aromas without the effects on the positive aromas associated with larger copper additions.

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REFERENCES

Azenha, M.; Vasconcelos, M.T. and Moradas-Ferreira, P. (2000) The influence of Cu concentration on ethanolic fermentation by *Saccharomyces cervisiae*. J. Biosci. Bioeng. 90(2):163-167.

Clark, A.C.; Grant-Preece, P.; Cleghorn, N. and Scollary, G.R. (2015) Copper(II) addition to white wines containing hydrogen sulfide: residual copper concentrations and activity. Aust. J. Grape Wine Res. 21:30-39.

Danilewicz, J.C. (2007) Interaction of sulfur dioxide, polyphenols, and oxygen in a wine-model-system: central role of iron and copper. Am. J. Enol. Vitic. 58:53-60.

Darriet, P.; Bouchilloux, P.; Poupot, C.; Bugaret, Y.; Clerjeau, M.; Sauris, P.; Medina, B. and Dubourdieu, D. (2001) Effects of copper fungicide spraying on volatile thiols of the varietal aroma of Sauvignon Blanc, Cabernet Sauvignon and Merlot wines. Vitis 40(2):93-100.

Godden, P. (2000) The use of copper sulphate in winemaking. Aust. N.Z. Wine Ind. J. 4:66-67.

Huang, C.; Huang, C. and Morehart, A.L. (1990) The removal of Cu(II) from dilute aqueous solutions by *Saccharomyces cervisiae*. Water Res. 24(4):433-439.

Liang, Q. and Zhou, B. (2007) Copper and manganese induce yeast apoptosis via different pathways. Molec. Biol. Cell 18(12):4741-4749.

Siebert, T.E.; Solomon, M.R.; Pollnitz, A.P. and Jeffery, D.W. (2010) Selective determination of volatile sulfur compounds in wine by gas chromatography with sulfur chemiluminescence detection. J. Agric. Food Chem. 58(17):9454-9462.

Spiropoulos, A.; Tanaka, J.; Flerianos, I. and Bisson, L. (2000) Characterisation of hydrogen sulfide formation in commercial and natural wine isolates of *Saccharomyces* . Am. J. Enol. Vitic. 51(3):233-248.

Viviers, M.Z.; Smith, M.E.; Wilkes, E. and Smith, P. (2013) Effects of five metals on the evolution of hydrogen sulfide during anaerobic storage of Chardonnay and Shiraz wines. J. Agric. Food Chem. 61(50):12,385-12,396.

Wang, J. and Chen, C. (2006) Biosorption of heavy metals by *Saccharomyces cervisiae*: A review. Biotechnol. Adv. 24.5:427-451.

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